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Development of a cobalt electrode for the determination of Phosphate in soil extracts and comparison with standard methods

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Development of a cobalt electrode for the determination of Phosphate in soil extracts and comparison with standard methods

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Abstract

A phosphate-sensitive cobalt electrode was evaluated in detecting orthophosphate ions (H_2PO_4^-) in ammonium lactate-acetic acid soil extracts. The dependence of the mixed potential of a cobalt electrode on H_2PO_4^- concentration was investigated via potentiometry. The mechanism of detection is based on the consumption of a surface cobalt (II) oxide layer to form $(\text{Co}_3(\text{PO}_4)_2)$, which leads to a concentration-dependent shift of the mixed potential. Two reference electrodes were evaluated: Ag/AgCl (3 M) KCl and a platinum (Pt) wire. A linear response was observed using both reference electrodes. However, application of a Pt wire quasireference electrode increased the linear dynamic response range of the detector from $10\text{--}10^3 \text{ mg L}^{-1}$ or $10^{-4}\text{--}10^{-1} \text{ M}$ (Ag/AgCl (3 M) KCl) to $0.1\text{--}10^5 \text{ mg L}^{-1}$ or $10^{-6}\text{--}10^1 \text{ M}$. In addition, the response time using the Pt wire was less than 5 minutes compared to a minimum of 10 minutes using Ag/AgCl (3 M) KCl. There was close agreement between the response of the phosphate-sensitive cobalt electrode with a standard colorimetric method. As dissolved organic substances can potentially interfere with electrochemical techniques, an investigation into the use of a nonpolar resin for decolorization and removal of organic matter in soil extracts was carried out and successfully employed. The phosphate-sensitive cobalt electrode was found to be a fast method for the analysis of soil extracts with high sensitivity and selectivity. It has the potential to be developed into a sensor for the *in situ* measurement of phosphate in various environmental matrices.

Key words: ammonium lactate-acetic acid extraction, potentiometry, phosphate determination, cobalt electrode, soil extract.

31 Introduction

32 Phosphorus (P) is one of the essential elements for plant growth and development
33 because P plays a vital role in the cell physiology and biochemistry of all living
34 organisms (Hanrahan et al. 2005; Adeloju 2013). Phosphorus naturally exists in either
35 organically or inorganically-bound forms, mostly as insoluble phosphate minerals
36 (Hanrahan et al. 2005; Adeloju 2013; Warwick et al. 2013). In soil extracts (water and
37 dilute salt solutions such as KCl and CaCl₂), P is usually found in the form of the
38 orthophosphates H₂PO₄⁻ and HPO₄²⁻ (0.01 – 3.0 mg L⁻¹) (Sims 2000; Warwick et al.
39 2013), which are the main readily available forms of P for plant growth. In rivers,
40 orthophosphate concentrations vary greatly from 0.02 – 1 mg L⁻¹. (Quintana et al.
41 2004; Warwick et al. 2013). In natural and waste waters orthophosphate
42 concentration varies from 0.2 - 10 mg L⁻¹, while in soil it varies from 0.2 - 50 mg kg⁻¹
43 (Warwick et al. 2013). In recent decades, a large increase in the use of phosphate-
44 containing fertilisers has resulted in increased concentrations of orthophosphate in
45 land runoff, which can lead to eutrophication. This process is regarded as one of the
46 major threats to the aquatic environment (Abowei et al. 2005). Thus, there is a need
47 for the development of improved orthophosphate detection techniques in order to
48 enhance our knowledge regarding the major sources of orthophosphate input to
49 water bodies and to improve our understanding of P-cycling in various environments.

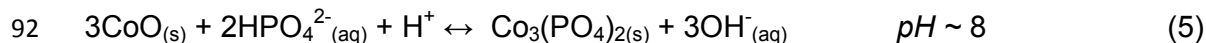
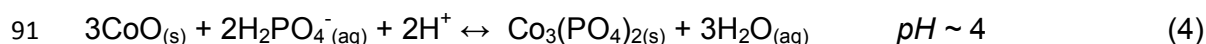
50 The methods used for the monitoring and management of environmental P, based
51 on its accurate determination in soil and water samples, were reviewed by Worsfold
52 et al (2005). Most often the Murphy and Riley (1962) colourimetric method is used to
53 quantify soluble reactive P because of its low limit of detection (≤ 0.01 mg L⁻¹)
54 (Murphy and Riley 1962; Motomizu 1983; Pierzynski 2005). For total P analysis
55 (inorganic and organic-bound P), samples can be acid digested and then analysed
56 via Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), with a
57 limit of detection of 0.03 mg L⁻¹ (Thompson and Walsh 2003; Pierzynski 2005). Ion-
58 Chromatography (IC) directly measures orthophosphate concentration with a limit of
59 detection of 0.002 mg L⁻¹ (Mckelvie et al. 1995). Orthophosphate has also been
60 determined using indirect techniques: precipitation with Ca²⁺ or Pb²⁺ in flow injection
61 analysis (FIA) (Midgley 1979; Chen et al. 1997) or using ion selective electrodes
62 (ISEs) (Marco et al., 1998; Vanstaden 1993; Xiao et al. 1995; Lee et al., 2009).
63 However, the indirect methods are often affected by matrix interferences due to the

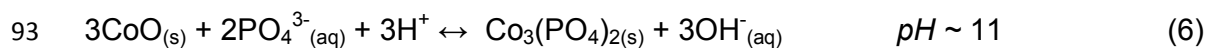
formation of Pb^{2+} and Ca^{2+} compounds with other anions; (Midgley 1986; Alexander and Koopetngarm 1987; Xiao et al. 1995; Berchmans et al. 2012; Warwick et al. 2013).

In recent years a number of methods have been reported for the electrochemical detection of orthophosphate (Xiao et al.1995; Meruva and Meyerhoff 1996; Adeloju 2013; Berchmans et al. 2012; Warwick et al. 2013). Many of these methods are discussed in a recent review (Berchmans et al. 2012). The potentiometric detection of orthophosphate using simple metal electrodes is a promising method of quantification. Although the potential for selectivity issues may be increased compared to biosensors, systems involving ion selective membranes and other indirect techniques (Berchmans et al. 2012), metal electrodes are cheap, simple and robust. For example, while salophen-based electrochemical sensors show good selectivity for HPO_4^{2-} down to 10^{-5} M with short response times, they have short lifetimes and require strict laboratory conditions (Berchmans et al. 2012; Kim et al.2008). Xiao et al (1995) reported the first comprehensive evaluation of a phosphate-sensitive electrode based on a redox reaction between cobalt and dissolved orthophosphates. The dynamic range was $1\text{--}1000\text{ mg L}^{-1}$ or $10^{-5}\text{--}10^{-2}$ M using 0.025 M potassium hydrogen phthalate as a buffer solution. The hypothesised mechanism for the phosphate-sensitive Cobalt electrode is dependent on the formation of an oxide layer (Eqs (1)- (3)) (Mervu and Meyerhoff (1996)



The cobalt oxide layer reacts with orthophosphates (Eqs (4) – (6)), the exact nature of which are dependent on pH. The potentiometric response given by these reactions can then be related to phosphate concentration because it affects the concentration of available CoO (Eq 1).





The formation of cobalt (II) phosphate in the reactions outlined (Eqs (4) – (6)) consumes cobalt (II) oxide, perturbing the equilibrium (Eq (3)). This equilibrium must be restored by further oxidation of cobalt from the electrode surface to form cobalt (II) oxide, which shifts the mixed potential. The measured mixed potential for this system is not a true equilibrium potential, but a steady state potential. It occurs when the anodic current (i_a) formed by the dissolution (oxidation) of the metal at the anode (Eq (1)), is equal in magnitude to the cathodic current (i_c) formed by the reduction of oxygen (Eq (2)) at the cathode ($|i_a| = |i_c|$). This results in the reaction having a net current of zero, but the reactions involved are still taking place at a measurable rate. The shift in potential is directly proportional to the logarithm of the phosphate concentration according to the Nernst equation. The electrode response also depends on ionic strength, buffer salts and sample stirring rate (Meruva and Meyerhoff 1996; Engblom 1999). Engblom (1999) applied the cobalt electrode to soil extracts, using ammonium lactate-acetic acid as extractant for available P, achieving a linear range 10–100 mg L⁻¹ or 10⁻⁴–10⁻³ M. However, this is not sufficiently sensitive for environmental applications because the concentration of orthophosphate in fertile arable soil extracts is usually between 0.01–3.0 mg L⁻¹ while for freshwater analysis concentrations of less than 0.1 mg L⁻¹ (UK environmental agency guidelines) need to be reached. A common matrix interference encountered in the analysis of soil extracts by electrochemical methods is the deposition of dissolved organic matter on the electrode surface. Dissolved organic matter can be reduced by washing extracts with carbon black; however, slight increases in P concentration have been noted due to the leaching of P from carbon black (Engblom 1999). For the electrochemical quantification of

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orthophosphate, an easy method for the removal of organic matter which does not influence the available P concentration is highly appealing and vital for accurate and reliable measurements.

In this study, the response of the phosphate-sensitive cobalt electrode in ammonium lactate-acetic acid soil extracts is investigated. The removal of organic matter using Amberlite XAD-4 resin is also introduced as an advantageous alternative to the aforementioned carbon black. The electrochemical sensor was also examined to improve its detection range. The electrochemical performance of the cobalt-based sensor was analysed in the presence of two difference reference electrode systems: a Pt wire (a single phase reference and so a ‘quasireference’ electrode) and an Ag/AgCl (3 M) KCl reference electrode (an electrode of the 2nd kind, three phases in equilibrium) and the detection range, response time and reproducibility of both methods were compared.

Materials and Methods

Cobalt electrode

The electrodes used in this work were fabricated from 99.99% pure cobalt rods (50 mm length, 2 mm diameter, 3.14 mm² cross sectional area, Goodfellow, Cambridge, UK). The rods were cut in half and each half was encased in a Polytetrafluoroethylene (PTFE) housing, leaving one third exposed as a point of contact for the multimeter (Figure 1). The surfaces of the electrodes were polished mechanically using a polishing wheel (Buehler Metaserv 2000 grinder/polisher, USA), fitted with abrasive polishing pads (Buehler, USA), alongside different grades of alumina powder (15 µm, 3 µm and 1 µm, Buehler micropolish, USA), prepared as aqueous suspensions. The electrodes were then washed with water and sonicated (Branson 1510 sonicator, USA). Each freshly polished electrode was then

143 conditioned for 2 hours by immersion in deionised water. Figure S1 (in supporting
144 information) shows the experimental setup. Two reference electrodes were used, an
145 Ag/AgCl (3 M) KCl reference electrode (MI-401, Microelectrodes Inc. USA), and a
146 platinum wire (Pt) of 99.9% purity (Goodfellow, Cambridge, UK).

147 For measurements involving the Ag/AgCl (3 M) KCl reference, the potential
148 difference between the two electrodes (cobalt working and Ag/AgCl (3 M) KCl
149 reference) was monitored using a digital multimeter (Metex M-3000, USA, input
150 impedance < 10 M ohms) during conditioning in deionized water until a stable value,
151 usually below -280 mV, was attained. The electrodes were then placed in buffer
152 solution (0.1 M ammonium lactate and 0.4 M acetic acid solution) until a new steady
153 state was established (~45 minutes). At this point, the electrode was ready for use.
154 During measurements, the electrode was immersed for an equilibration period of 10
155 minutes in the sample/ orthophosphate standard solution before the potential was
156 recorded. This requirement was determined based on the observed response times
157 of the sensor when analyzing orthophosphate standard solutions of concentrations
158 below 1 mg L⁻¹ (10⁻⁵ M). The stability of the electrode in the ammonium lactate-acetic
159 acid solution was also investigated for a period of 7 days.

160 The platinum wire quasireference electrode was first calibrated to determine its
161 potential against a true reference electrode. This was determined against an
162 Ag/AgCl (3 M) KCl reference electrode in ammonium lactate-acetic acid solution
163 using the multimeter and the data is given in the supporting information (Table S1).
164 The experimental setup (Figure S1) was slightly modified when using the Pt wire.
165 The potential difference between the two electrodes (cobalt and Pt wire), was
166 monitored during the deionized water conditioning until a stable potentiometric
167 response (below -400 mV) was reached. The electrode was placed in buffer solution

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(0.1 M ammonium lactate and 0.4 M acetic acid solution) until a new steady state was established (~45 minutes). Before being used, the Pt wire quasireference was sterilised on a Bunsen burner and left to cool and equilibrate in the solution for 5 minutes.

For measurements involving both reference electrodes (Ag/AgCl (3 M) KCl and Pt wire), the solutions were stirred constantly using a magnetic stirrer (HANNA instruments Hi-190M, UK). The cobalt electrode was always rinsed with deionized water after each reading to extend the lifetime of the sensor by reducing interferences that could occur due to accumulation of phosphate on the surface of the electrode. The electrodes were freshly polished and conditioned after each batch of samples had been analysed. Prolonged use without polishing, particularly in the analysis of soil extracts, lead to a lower sensitivity and longer response time. All calibration measurements were carried out in triplicate and the averages of these measurements were used to plot the calibration curve.

The cyclic voltammograms of the phosphate layer formation were recorded using an auto lab electrochemical analyser (Autolab potentiostat PGSTAT30). A three electrode system was used: an Ag/AgCl (3 M) KCl reference, a Pt wire counter and the cobalt working electrode. The electrolyte consisted of a 0.1 M solution of potassium dihydrogen phosphate (mono-basic) in 0.1 M ammonium lactate and 0.4 M acetic acid buffer (pH 3.75). The experiment was performed at a scan rate of 100 mV s⁻¹ over a potential range of -800 mV – 800 mV for 50 scans. The electrolyte was degassed by sparging it with Nitrogen (N₂) for 10 minutes prior to the experiment to remove dissolved oxygen (O₂).

Sample Collection and Preparation.

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3 192 Soil samples (n=5) were collected in June 2012 from the Henfaes Research Centre,
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5 193 (Abergwyngregyn, United Kingdom) from the following coordinates; (53°14'28.08N,
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7 194 4°01'17.97" W). The site has been a permanent pasture over the last 11 years and
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10 195 has constantly been grazed by sheep. Samples were collected using an auger
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12 196 (Eijkelkamp, Holland) up to a soil depth of 30 cm. The soil cores (inner diameter, 8
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14 197 cm), were split into two sections: Surface sA (0 cm - 15 cm) and subsurface sB (15
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16 198 cm - 30 cm). Each sample was air-dried, passed through a 2 mm sieve and stored
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18 199 for analysis.

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21 200 Compost samples (T1 to T4) were collected from the Treborth botanical gardens,
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23 201 (Bangor, United Kingdom), and were used to compare the capability of absorbing
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25 202 organic matter using Amberlite XAD-4 (Sigma Aldrich, UK) and carbon black (Fisher
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27 203 scientific, UK).

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31 32 205 **Sample treatment and P extraction**

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34 206 Soil pH values were measured with an Orion (Boston, USA) 420A pH meter.
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36 207 Samples were prepared in a 1:2.5 w/v soil to deionised water ratio and were stirred
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38 208 (HANNA instruments Hi-190M, UK) for 15 minutes prior to the measurement.
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40 209 Organic matter was determined by the loss of ignition method, heating 10 g of
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42 210 sample to 450 °C in a Muffle furnace (Carbolite, UK) for 4 hours.

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46 211 The nitric acid (HNO₃) digestion used for total P determination was based on a
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48 212 modified procedure recommended by the United States Environmental Protection
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50 213 Agency (USEPA, Method 3050, 1989). One gram of soil was placed in a 100 mL
51
52 214 pyrex digestion tube and a pre-digestion step was run at room temperature for 16
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54 215 hours with 10 mL nitric acid (Sigma Aldrich reagent grade, 70%). Then, the
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56 216 suspension was digested at 120 °C for 4 h, in a Stuart SBH 200D13 digester (Bibby

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scientific UK). The obtained suspension was then filtered and diluted to 100 mL with deionised water, before being stored in polyethylene bottles at 4 °C for analysis by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

Ammonium lactate-acetic acid extraction for available and total dissolved P was based on the protocols of Engblom (1999). Five grams of the soil was weighed into a 250 mL Erlenmeyer flask and mixed with 100 mL of 0.1 M ammonium lactate and 0.4 M acetic acid solution (pH 3.75). This was shaken using a Thermo Scientific Max Q Shaker, UK for 90 minutes, filtered and stored at room temperature for analysis by Murphy Riley (1962) colourimetry, ICP-OES and electrochemical methods.

The four compost samples (T1 to T4) were analysed for extractable P (Olsen P in mg kg⁻¹). For the carbon black method, the removal of organic matter from the compost extracts was carried out by adding 0.5 g of carbon black to 50 mL of an extract (T1 to T4) and shaking it for 15 minutes. For the resin method, 10 g of wet resin (corresponding to ca. 4 g dry resin) was added to 50 mL of an extract (T1 to T4) and shaken for 20 hours. After filtration through Whatman no.42 filter paper, the orthophosphate concentrations of the extracts were determined using Murphy and Riley (1962) colourimetry.

Reagent and solutions

Deionised water (Elga option 4 water purifier, USA) was used for the preparation of samples, extracts and standard solutions. All other chemicals were of analytical-reagent grade such as HNO₃ (Sigma Aldrich reagent grade, 70%). All phosphate standards used for the electrochemical analysis were prepared in ammonium lactate-acetic acid solution daily from a monobasic potassium phosphate stock solution (Sigma Aldrich, UK purity of >98%) across a concentration range of 0.001 –

10⁵ mg L⁻¹ or 10⁻⁸ – 10¹ M. Method interferences caused by contaminants in deionised water, reagents, glassware, and other sample processing was reduced by washing all glassware with 2% HCl, rinsing with deionised water and allowing it to dry before use.

Instruments

A Varian Agilent 710-ES model ICP-OES with a Varian SPS-3 auto sampler was used for P determination of the soil extracts. The operating parameters of the instrument were: Wavelength: 213.618 nm, RF Generator power: 1.2 kW, plasma argon flow: 15.0 L min⁻¹, auxiliary argon flow: 15.0 L min⁻¹, nebulizer pressure; 200Kpa, nebulizer argon flow; 0.75 L min⁻¹, replicate time; 5s, sample uptake: 5 mL, rinse time 30s, pump rate: 12 rpm.

A Jasco V550 UltraViolet Visible Spectrophotometer (Maryland court, USA) was used for the colorimetric analysis using the Murphy Riley method. Measurements were carried out at a wavelength of 880 nm and path length of 5 cm.

Data Analyses and Quality Control

All soil results were reported based on their air-dried weight at room temperature (25°C). Statistical differences among means were evaluated using student t-test (paired two sample for means), ANOVA-single factor and F- Test. All analytical results are means of three replicate analyses with standard errors less than ± 5% of the mean value.

Results

Phosphate-Sensitive Cobalt Electrode: Calibration and Stability

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Cyclic voltammetry was applied to the cobalt electrode in the presence of orthophosphate. The cyclic voltammograms (Figure 1) show the oxidation of cobalt to cobalt (II) oxide (Co^0 to Co^{2+}), which leads to the formation of cobalt (II) phosphate in the presence of orthophosphate (Meruva and Meyerhoff 1996). This is recognisable by the anodic processes commencing between -200 to -100 mV vs. Ag/AgCl (3M) KCl on the forward scans. On the reverse scans, no significant cathodic processes are observed, as expected (Meruva and Meyerhoff 1996). This indicates that the formation of Co^{2+} is irreversible within the potential range. The anodic peak therefore decreases in magnitude with successive scans as the electrode surface becomes increasingly coated with cobalt (II) phosphate, effectively reducing the electrode surface area (Figure 1). This also has the effect of shifting the anodic processes to more positive potentials with successive scans.

The potentiometric response of the cobalt electrode coupled to an Ag/AgCl (3 M) KCl reference electrode to different concentrations of phosphate standards was also examined (Figures S2 and S3 in supporting information). To gain insight into the lifetime of the sensor, the detection range of the electrode calibrated in the same beaker, several times over 7 days (stored under air without polishing between runs) was carried out. After three days, a change in the response of the electrode at low concentrations was observed (Figure S2).

The original linear characteristics were retained after polishing and sonicating of the electrode. Since the electrode had been exposed to air, the cobalt surface may have been oxidized and covered by particles and precipitates. These particles were removed by the polishing and sonication of the surface of the electrode. Coupled to a Pt wire quasireference electrode, the cobalt electrode showed a linear response to varying orthophosphate concentration. The modified system exhibits improved

288 sensitivity towards phosphates ions, resulting in an increase in the linear range of
289 detection to $0.1 - 10^5 \text{ mg L}^{-1}$ or $10^{-6} - 10^1 \text{ M}$ (Figure S2). Repeatability was also
290 observed for the low concentration orthophosphate standards.

291 **Phosphorus Quantification in Soil Extracts: Comparison between the cobalt**
292 **electrode coupled to an Ag/AgCl (3 M) KCl Reference Electrode and**
293 **Colourimetry**

294 Ammonium lactate-acetic acid soil extracts were chosen to test the phosphate-
295 sensitive cobalt electrode as they provide higher concentrations of orthophosphate
296 compared to freshwater. Table 1 shows the total P concentration of all ten soil
297 samples, which ranged from $384 - 1189 \text{ mg kg}^{-1}$ (Measured using ICP-OES on nitric
298 acid extracts). The organic matter content ranged from $2.9 - 7.6 \%$ and pH in water
299 ranged from $5.4 - 8.0$. The Murphy and Riley colourimetric technique is the most
300 accepted standard method for the measurement of orthophosphate in soil extracts
301 (Worsfold et al. 2005). The cobalt electrode coupled to a Ag/AgCl (3 M) KCl
302 reference electrode showed no significant differences compared to the colourimetric
303 P determination for eight of the ten soil extracts while samples s3A and s3B showed
304 a significantly lower concentration ($p = 0.02$). This deviation does not appear to be
305 linked to total P concentration, organic matter content, or pH (Table 1), indicating
306 that the sensor is potentially susceptible to other matrix interferences.

307 The actual solution orthophosphate concentrations in the soil extracts ranged from
308 $0.3 - 1.9 \text{ mg L}^{-1}$. As the actual orthophosphate concentrations were below the linear
309 detection range for the Ag/AgCl (3 M) KCl reference electrode (Figure S2), the
310 results reported (Table 1) were calculated based on the lower and upper limiting
311 standards bracketing these concentrations (Engblom 1999).

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In order to evaluate the sensitivity of the cobalt electrode coupled to a Pt wire quasireference electrode as a system for detecting orthophosphate concentrations in ammonium lactate-acetic acid soil extracts, five soil samples were tested and the data were compared to those obtained using the Ag/AgCl (3 M) KCl reference electrode method and colourimetry. Results (Figure 2) showed no significant differences compared to the colourimetric P determination for two out of the five samples but significantly lower concentrations for the other three samples ($p = 0.04$ s2A, $p = 0.03$ s2B and $p = 0.02$ s4B). Although the platinum quasireference method provides a wider linear range of detection compared to the Ag/AgCl (3 M) KCl reference electrode method for standard solutions, it appears that it may be more susceptible to matrix interferences in natural media, potentially due to the adsorption of organic compounds onto the surface of the platinum electrode.

Organic matter influence on P determination

Four compost extracts (T1-T4) high in organic matter content were used to compare carbon black and amberlite XAD-4 for the removal of dissolved organic matter. From loss on ignition analysis the organic matter content was determined as T1 = 96 %, T2 = 95 %, T3 = 93 % and T4 = 96 %.

Results from the ANOVA-single factor analysis revealed that there was a significant difference between the treatments ($p < 0.05$) within each sample. Treatment of the compost extracts with non-polar resin resulted in a noticeable change in colour. Further analysis to differentiate between each treatment, using the Fisher test (F-test two sample for equal variance, ($\alpha = 0.05$)) revealed that for sample T3 there was no significant difference between no-treatment, carbon black and resin treatment ($p > 0.05$). For samples T1, T2, there was no significant difference between no-treatment and resin treatment, but a significant increase in P concentration after carbon black

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3 337 treatment was observed ($p < 0.05$). This could be due to the fact that phosphoric acid
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5 338 is often used in the preparation of carbon black/ activated charcoal. T4 showed a
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7 339 significant difference between the three treatments, with lowest values found in resin
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9 340 treatment and highest in the carbon black treatment (Figure 4).

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11 341 Overall, the results showed that the non-polar resin adsorbed organic matter from
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13 342 extracts without increasing the P concentration, while carbon black treatment for all
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15 343 samples added P to the compost extracts. However, this effect seems to be less
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17 344 relevant in samples with lower organic matter content (i.e. T3) but leads to significant
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19 345 differences in P results in samples with higher organic matter content (i.e. T4).

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21 346 The non-polar resin was applied to the ten surface (s1A–s5A) and sub-surface (s1A–
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23 347 s5B) soil extracts and P was determined using colourimetry (C) and the phosphate-
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25 348 sensitive cobalt electrode coupled to the Ag/AgCl (3 M) KCl reference electrode
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27 349 method (P). The results obtained with the two different methods before and after
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29 350 resin treatment are shown in Table 2.

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35 351 Results from the electrode measurements showed that there was a 21–55%
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37 352 decrease in orthophosphate concentration across the surface soil while a 16–56 %
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39 353 significant decrease was observed across the sub-surface soils ($p = 0.03$). It was
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41 354 also noticed that the electrode attained a steady potential earlier (< 5 min) after resin
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43 355 treatment. The results from the colourimetric analysis also showed a 8–26 %
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45 356 reduction in orthophosphate concentration across the surface soils, while the sub-
46
47 357 surface soils showed a 24–42% significant reduction ($p = 0.004$) (Table 2).

358 Discussion

359 The formation of a cobalt (II) phosphate layer in the presence of orthophosphate was
360 confirmed by cyclic voltammetry. The anodic peak decreased with an increasing
361 number of scans because of the accumulation of cobalt phosphate on the surface of

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the electrode, which forms a layer preventing the oxidation of Co^0 to Co^{2+} in successive scans (Meruva and Meyerhoff 1996; Engblom. 1999).

Using the cobalt electrode coupled to an Ag/AgCl (3 M) KCl reference electrode in standards prepared in ammonium lactate-acetic acid solution, the linear detection range attained was consistent with that reported by Engblom (1999). However, this is still higher than most orthophosphate concentrations routinely measured in freshwater (Sims 2000; Warwick et al. 2013). The maximum permissible orthophosphate concentration in river water according to Environment Agency guidelines in the UK is $0.04\text{--}0.12\text{ mg L}^{-1}$ which implies that the limit of quantification ought to be one tenth of this: 0.01mg L^{-1} . Our results also showed that stirring the solution during measurement did not adversely affect the calibration curve, which maintained its linear relationship with orthophosphate concentrations. This may make this system advantageous for applications in flowing conditions for *in situ* measurements. This result also confirmed previous observations by Engblom (1999) and Lee et al (2009) who demonstrated that the stirring rate did not affect the sensitivity and stability of the electrode response. Using the Pt wire in place of the Ag/AgCl (3 M) KCl reference electrode led to an increase in the detection range of the electrode by four orders of magnitude compared to that reported by Engblom et al. of between $\sim 10\text{--}10^3\text{ mg L}^{-1}$ ($10^{-4}\text{--}10^{-1}\text{ M}$) (Engblom 1999). A simple Pt wire could be considered a good replacement for the Ag/AgCl (3 M) KCl and calomel reference electrodes that often have a limited range of applications due to the liquid junctions in these cells, which are not stable and often prone to degradation. Platinum wire has also been used as a solid state reference electrode because of its rapid response and physical form, which yields reproducible results as a reference electrode (Chaston 1974; Kasem and Jones 2008). The proposed method using a Pt

387 wire exhibits a lower dynamic linear potential response in the concentration range of
388 $0.1\text{--}10^5 \text{ mg L}^{-1}(10^{-6} - 10^1 \text{ M})$. This range is wider and extends to lower
389 concentrations compared to most other cobalt based phosphate sensors previously
390 described: (Berchmans et al. 2012): $1\text{--}1000 \text{ mg L}^{-1} (10^{-5} - 10^{-2} \text{ M})$ reported by Xiao
391 et al. (1995), $10\text{--}1000 \text{ mg L}^{-1} (10^{-4} - 10^{-2} \text{ M})$ by Meruva and Meyerhoff (1996), 1--
392 $10000 \text{ mg L}^{-1} (10^{-5} - 10^{-1} \text{ M})$ by Lee et al. (2009) and $1 - 500 \text{ mg L}^{-1} (5 \times 10^{-3} - 10^{-5}$
393 $\text{M})$ by Chen et al. (1997).

394 Total extractable P in ammonium lactate-acetic acid soil extracts was quantified
395 using ICP-OES (both organically and inorganically-bound P) (Mckelvie et al. 1995),
396 as was reviewed by Pierzynski et al. (2005). Bioavailable inorganic P was quantified
397 colourimetrically using the blue reduced phosphor-molybdenum complex method
398 established by Murphy and Reily (1962) - the standard method for the determination
399 of orthophosphates in soil extracts. The differences between the two methods were
400 proportionally larger when the extractable P concentration in the soil was below 20
401 mg kg^{-1} . The largest difference between the two methods was observed for sample
402 s4a, which features the highest organic matter content and so the largest content of
403 P not present as bioavailable orthophosphate. Potentiometry on the other hand,
404 gave lower values. The largest difference was observed in samples s3A and s3B,
405 where the electrode measurements were lower compared to colourimetry and ICP-
406 OES values (Table 1). Thus, resulting in lower values than the actual
407 orthophosphate concentration in the extracts. This occurred likely as a result of the
408 presence of organic substances and iron in the extracts not removed during the
409 carbon black treatment (Table 2), which could have led to slight drifts towards more
410 positive potentials during measurements. This is supported by the very low organic
411 matter removal efficacy shown by s3A and s3B (Table 2). This hypothesis is also

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412 supported by Engblom (1998), who investigated the effect of organic matter and iron
413 on the electrode measurements. He reported that the presence of other competing
414 oxidizing substances (i.e. iron) in the soil extracts could compete with the reduction
415 of oxygen, if present, causing drifts towards higher potential values. Thereby
416 resulting in the underestimation of the actual orthophosphate concentration in the
417 soil extracts.

418 The standard method for the determination of orthophosphates in soil extracts using
419 the reduced phosphor-molybdenum blue complex and colourimetry has been
420 established by Murphy and Reily (1962). It is highly sensitive in detecting inorganic P
421 and orthophosphate ions (Limit of detection = 0.01 mg L⁻¹) and a stable complex is
422 formed in the presence of antimony. However, the antimony-phospho-molybdate
423 complex can be reduced to an intensely blue-colored complex (required to measure
424 absorbance) by ascorbic acid within 30 minutes. Barium, lead, silver and silica might
425 interfere with the reaction by forming precipitates overestimating orthophosphate
426 concentrations (Murphy and Reily 1962). Coloured humic material in soil and water
427 extract can influence the absorption and is often removed prior to measurement. In
428 addition, the acidic nature of the reagent might lead to the overestimation of
429 orthophosphates in soil extracts (Pierzynski et al. 2005; Worsfold et al. 2005.), due to
430 hydrolysis of weakly bonded inorganic or organic-condensed P species (Mckelvie et
431 al. 1995).

432 This is not the case with the phosphate sensitive cobalt electrode, and its simple
433 instrumentation and low fabrication cost, making it an attractive analytical tool
434 suitable for a wide variety of applications. Apart from its numerous advantages such
435 as increased opportunity for miniaturisation, and greater durability, it is not
436 dependent on colouring reagent, less prone to interferences which affect

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3 437 colourimetry and can analyse lower P concentrations. This was confirmed by the
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5 438 small difference in P concentration between the techniques shown by samples s1A
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7 439 and s1B, which also contained the lowest concentration of dissolved organic matter
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9 440 as shown in Table 1.

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12 441 The difference in solution orthophosphate values between the Pt wire quasireference
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14 442 and the Ag/AgCl (3 M) KCl electrode could also have been due to the presence of
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16 443 dissolved organic compounds in the soil extracts (Table 1), which have been
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18 444 reported to cause drifts in potential readings in soil extracts (Engblom 1999). In order
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20 445 to try and reduce this effect on the electrode a resin amberlite XAD-4 was used and
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22 446 shown to be able to adsorb organic matter. Unlike carbon black, the resin amberlite
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24 447 XAD-4 did not increase the P concentrations in the soil extracts.

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28 448 The phosphate sensitive cobalt electrode has already been applied in real
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30 449 applications

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33 450 (Engblom, 1998; Chen et al., 1997) and has proven successful for the measurement
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35 451 of orthophosphate in ammonium lactate-acetic acid extracts of soils (Engblom 1998).

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37 452 The suitability of the proposed method using the Pt wire quasireference electrode,
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39 453 makes it an attractive tool suitable for a wide variety of applications. The potential of
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41 454 the cobalt electrode coupled to a Pt wire for field-based measurements provides a
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43 455 versatile and indeed potentially invaluable screening option for measuring
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45 456 orthophosphate ions. Interest in the use of *in-situ* measurements originate from a
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47 457 need to provide a quick on-site assessment. Thus, alleviating much of the costs, time
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49 458 delays and loss of sample integrity associated with standard laboratory-based
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51 459 analysis which can be prone to interferences. The proposed method provides a quick
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53 460 and quantitative method for field measurements and can also be carried out by non-
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55 461 experts. However, going by the established history of potentiometric sensors, they
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have been reported to be prone to errors, resulting in significant drifts in potentials when measurements of very low concentrations are involved. However, despite the Pt wire set up having a lower resolution, it provides a viable platform for *in-situ* monitoring of phosphate and for trace analysis. For this to be feasible issues of matrix interferences still need further investigation.

Conclusions

The two reference electrode (Ag/AgCl (3 M) KCl and Pt wire) performed linearly and showed high sensitivity towards orthophosphate standards. However, the Pt wire quasireference electrode method gave a faster response time (less than 5 minutes) and a much wider dynamic detection range (0.1 – 10⁵ mgL⁻¹). It also showed good reproducibility with orthophosphate standards. Further investigation of the cobalt electrode coupled to an Ag/AgCl (3 M) KCl reference showed that its simplicity makes it an alternative to spectroscopic methods such as Ultraviolet Visible Spectroscopy and ICP-OES for the determination of P in soil extracts. Because of the good selectivity shown by the electrode, the results suggest that it gives a more reliable interpretation of the actual concentration of phosphate in the soil extracts, making it an attractive analytical tool for the direct determination of orthophosphate. Although the Pt wire method gave a better range when analysing standards, when tested on soil extracts, due to the complex matrices involved and interferences caused by the presence of organic matter, slight drifts in electrode response were noticed. Interferences due to the presence of organic substances in the extracts were reduced to a large extent by the use of a hydrophobic resin (Amberlite XAD-4), which was more reliable in both the removal of organic matter, and also in the indirect removal of organically bound P from extracts, compared to carbon black.

These preliminary results indicate that the use of the Pt wire as a reference electrode in the experimental set up is promising. A wide dynamic linear range of detection can be obtained: $0.1 - 10^5 \text{ mg L}^{-1}$. This is a significant improvement over some more complex potentiometric cobalt based phosphate sensors reported in the literature, which display linear responses within $1 - 10^4 \text{ mg L}^{-1}$ (Xiao et al. 1995; Meruva and Meyerhoff 1996; Kim et al. 2008; Lee et al. 2009; Chen et al. 1997; Berchmans et al. 2012). The technique has considerable potential for use in fresh water analysis, where organic matter influence would likely be minimal. Although this improvement is promising, further investigation is still needed to lower the limit of quantification by a further order of magnitude (to ca. 0.01 ppm) for accurate monitoring of orthophosphate levels in natural waters that are not yet subject to eutrophication (Quintana et al. 2004; Warwick et al. 2013, UK Environment Agency, 2012).

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Table 1. Properties of the soils ((A) soil surface 0-15 cm depth, (B) subsurface 15- 30 cm depth) and the P concentration as mg kg^{-1} of nitric acid digested extracts for total P and ammonium lactate-acetic acid extracts determined by three different analytical techniques. Electrochemical quantification was done with the Ag/AgCl (3M) KCl reference electrode as the average of triplicate measurements \pm the standard deviation.

Soil	pH (H_2O)	Organic matter (%)	Total P (mg kg^{-1})	Ammonium lactate-Acetic Acid soil extract (mg kg^{-1})		
				Inductive Coupled Plasma Optical Emission Spectroscopy	Colourimetry	Cobalt Electrode
s1A	8.0	2.9	417.5 ± 0.5	21.4 ± 0.10	19.8 ± 0.03	18.4 ± 4
s1B	7.9	2.6	383.5 ± 6.5	13.7 ± 0.03	11.0 ± 0.01	10.5 ± 2
s2A	5.5	5.1	546.6 ± 5.2	9.8 ± 0.03	7.0 ± 0.20	6.4 ± 4
s2B	5.6	3.9	439.3 ± 7.4	5.1 ± 0.03	5.0 ± 0.06	4.8 ± 2
s3A	6.0	6.0	988.4 ± 9.1	49.1 ± 0.20	47.2 ± 0.04	19.4 ± 4
s3B	6.3	5.4	878.6 ± 5.7	38.8 ± 0.01	34.2 ± 0.08	12.2 ± 5
s4A	6.0	7.6	1188.5 ± 4.1	87.9 ± 0.01	74.4 ± 0.30	60.4 ± 14
s4B	5.4	6.5	860.7 ± 2.7	40.9 ± 0.02	38.2 ± 0.24	37.2 ± 10
s5A	5.6	6.1	1042.5 ± 5.5	13.5 ± 0.01	10.9 ± 0.40	9.9 ± 2
s5B	5.8	5.6	790.5 ± 3.1	9.4 ± 0.03	5.5 ± 0.02	4.8 ± 3

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Table 2: Determination of orthophosphate (mg kg^{-1}) in the ammonium lactate-acetic acid soil extracts by (C) colourimetry and (P) potentiometry with the phosphate-sensitive cobalt electrode coupled to a Ag / AgCl/3M KCl reference electrode as the average of triplicate measurements \pm the standard deviation.

Surface Soil	Treatment			
	Colorimetry (C)		Potentiometry (P)	
	No Resin	Resin	No Resin	Resin
	mg kg^{-1}			
s1A	19.8 ± 0.03	15.4 ± 0.04	18.4 ± 4	10.9 ± 2
s2A	6.5 ± 0.3	5.1 ± 0.01	6.4 ± 4	4.34 ± 2
s3A	46.6 ± 0.7	40.9 ± 0.06	19.4 ± 4	14.7 ± 5
s4A	73.5 ± 0.4	68.8 ± 0.01	60.4 ± 14	35.8 ± 10
s5A	9.7 ± 0.5	8.96 ± 0.03	8.9 ± 2	4.3 ± 1

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Sub-Surface Soil	Colorimetry (C)		Potentiometry (P)	
	No Resin	Resin	No Resin	Resin
	mg kg ⁻¹			
s1B	10.6 ± 0.02	7.9 ± 0.3	10.5 ± 2	6.5 ± 5
s2B	5.1 ± 0.1	3.7 ± 0.02	4.8 ± 2	3.0 ± 1
s3B	34.3 ± 0.08	19.7 ± 0.02	12.2 ± 5	10.9 ± 4
s4B	38.2 ± 4	29 ± 0.02	37.2 ± 10	15.9 ± 3
s5B	5.5 ± 0.4	3.7 ± 0.02	4.8 ± 3	2.1 ± 2

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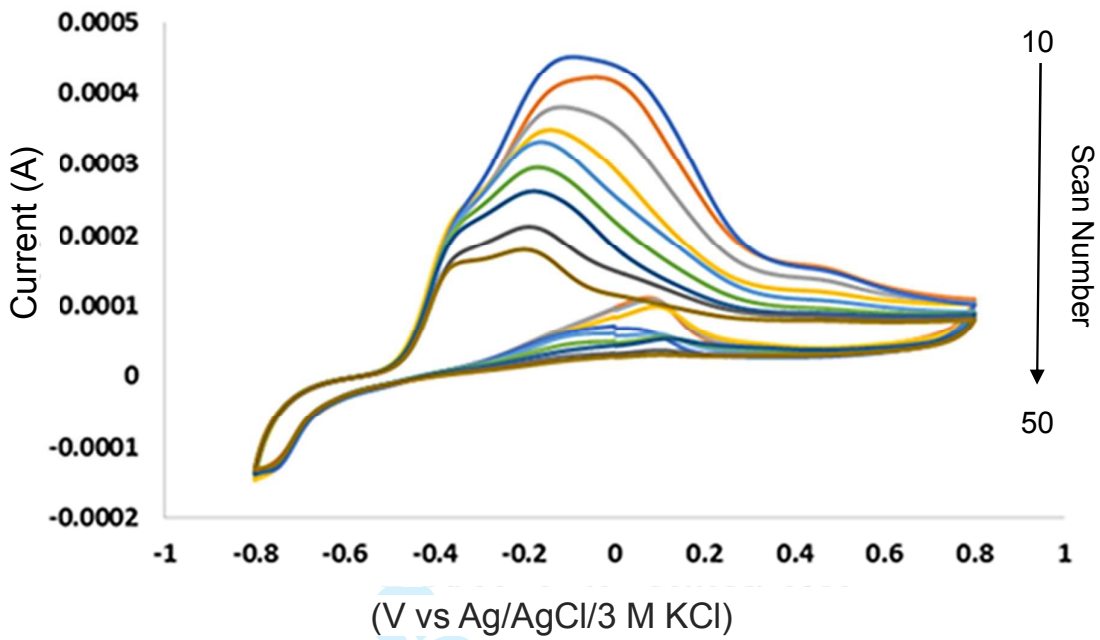


Figure 1. Cyclic voltammogram of the cobalt electrode with Ag/AgCl (3M) KCl reference and Pt wire as counter electrode in 0.1M KH_2PO_4 recorded with scan rate of 0.1 mV s^{-1} in the potential range of -800 mV to 800 mV showing 10 to 50 scans.

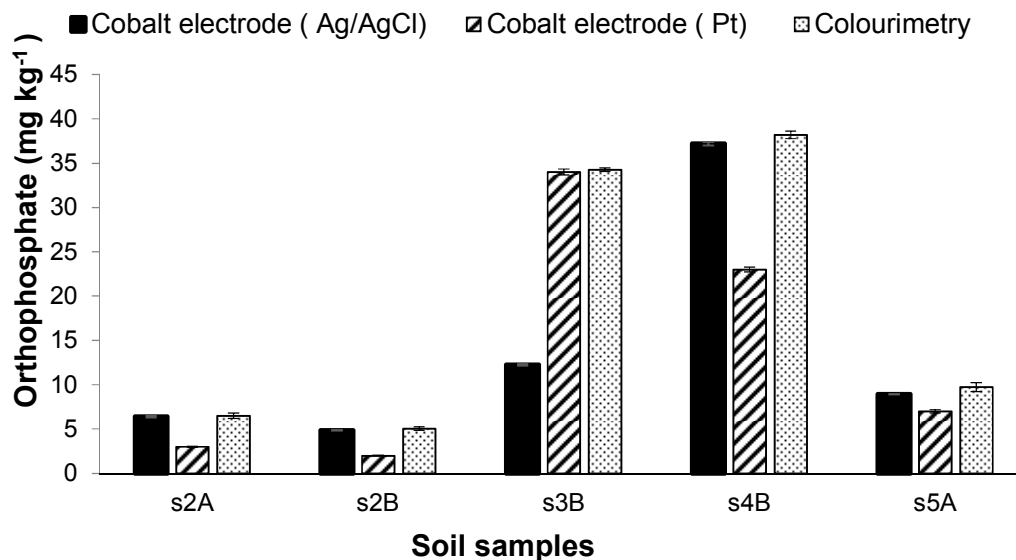


Figure 2. Comparison between the orthophosphate concentrations (mg kg⁻¹) in ammonium lactate-acetic acid soil extracts (pH=3.75) determined by cobalt electrode coupled to an Ag/AgCl (3M) KCl reference electrode (black bars), cobalt electrode coupled to a Pt wire quasireference electrode (shaded horizontal bars) and colourimetry (shaded spotted bars). (n=3, error bar = standard deviation)

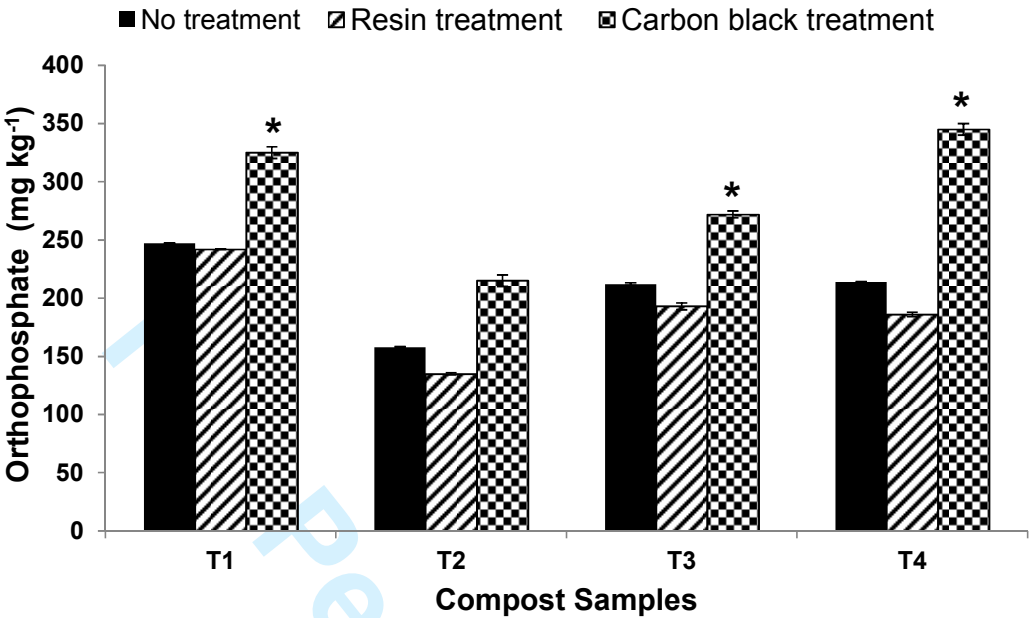


Figure 3. Orthophosphate concentrations in compost extracts (mg/ kg) determined by colorimetry with no treatment (black bars), Amberlite XAD-4 resin treatment (striped bars), and carbon black treatment (checkered bars) (n=3). *Indicates a significant difference (p < 0.05) between carbon black treatment and no treatment

Development of a cobalt electrode for the determination of Phosphate in soil extracts and comparison with standard methods

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Supplementary Information

- 1) Figure S1. Schematic of the Cobalt electrode
- 2) Table S1. Calibration data for the Pt quasireference electrode.
- 3) Figure S2. Calibration curve of a cobalt electrode response to the \log_{10} of orthophosphate standards dissolved in ammonium lactate-acetic acid solution (pH 3.75) using an Ag/AgCl / (3M) KCl reference electrode taken for a period of 7days.
- 4) Figure S3. Calibration curve of a cobalt electrode response to \log_{10} of orthophosphate standards dissolved in ammonium lactate-acetic acid solution (pH 3.75) using an Ag/AgCl (3M) KCl reference electrode and a Pt wire quasireference electrode.

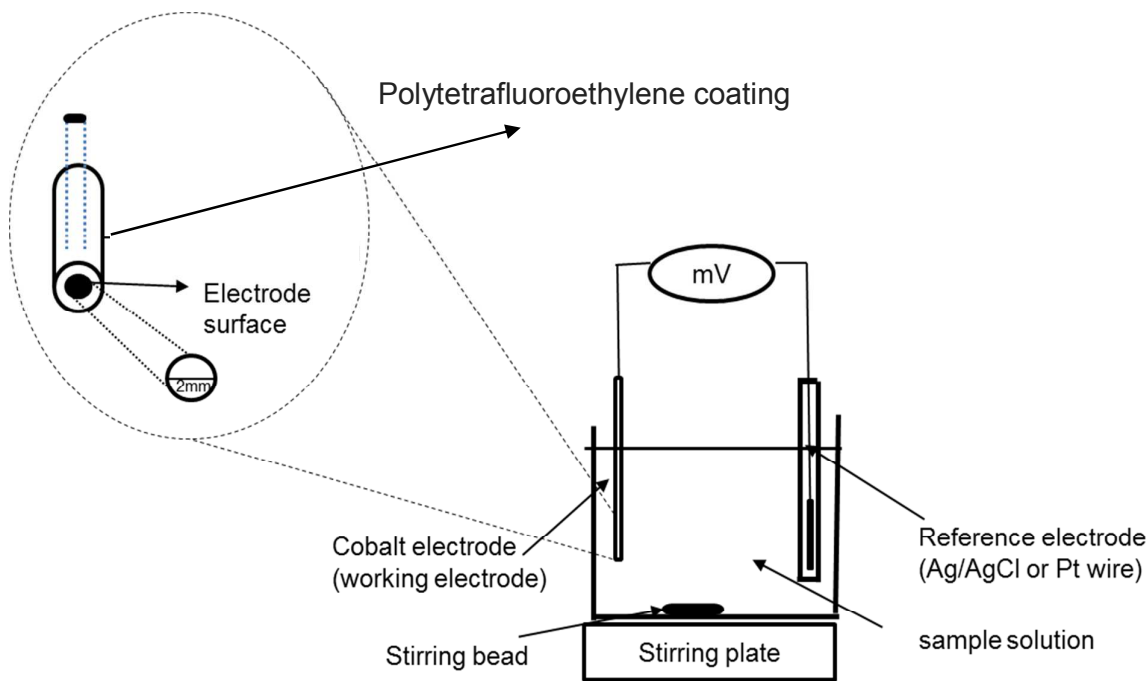


Figure S1. Schematic of the Cobalt electrode.

Table S1. Calibration data for the Pt quasireference

	Pt wire	SCE	SHE
Potential vs Pt wire	0.00 V	-0.38 V	-0.59 V
Potential vs SCE	0.38 V	0.00 V	-0.21 V
Potential vs SHE	0.59 V	0.21 V	0.00 V

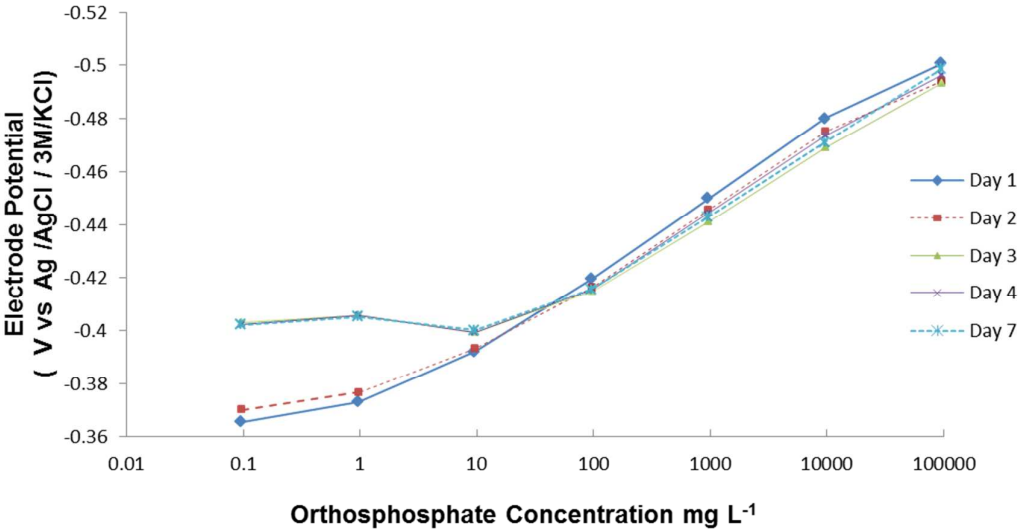


Figure S2. Calibration curve of a cobalt electrode response to the \log_{10} of orthophosphate standards ($0.1 - 10^5 \text{ mg L}^{-1}$) dissolved in ammonium lactate-acetic acid solution (pH 3.75) using an Ag / AgCl / (3M) KCl reference electrode taken for a period of 7 days.

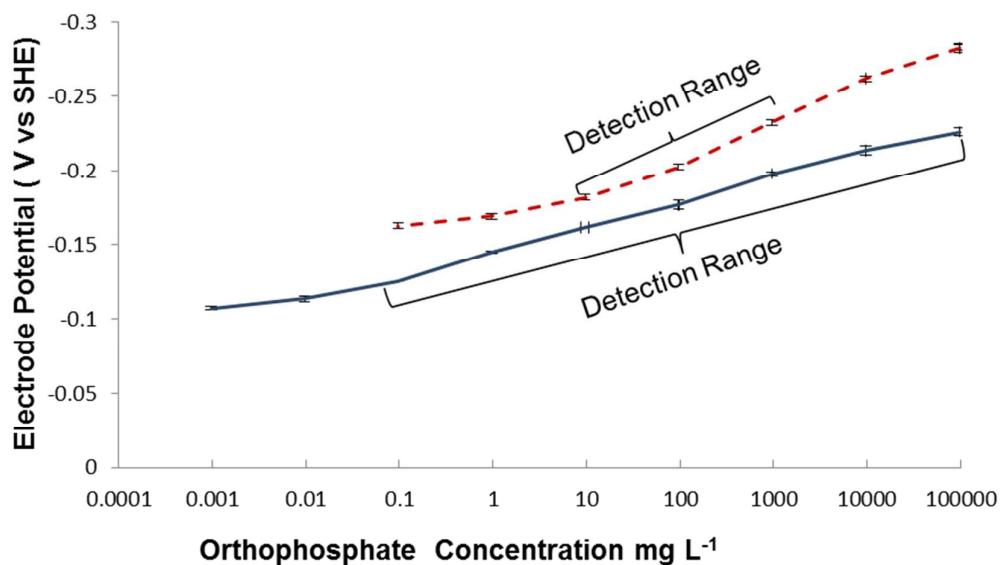


Figure S3. Calibration curve of a cobalt electrode response to \log_{10} of orthophosphate standards ($0.001 - 10^5$ mg L⁻¹) dissolved in ammonium lactate-acetic acid solution (pH 3.75) using an Ag / AgCl (3M) KCl reference electrode (dashed lines) and a Pt wire quasireference electrode (solid line).